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## Chloroplast replication and growth in tobacco

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## SUMMARY AND CONCLUSIONS

1. The greening and the growth of chloroplasts as induced by light has been investigated in leaf discs from etiolated tobacco leaves in sterile culture.

2. On a medium containing salts after Murashige and Skoog plus sucrose, chlorophyll synthesis proceeds very slowly during the first day after the transfer from darkness to light. Thereafter the rate of chlorophyll synthesis increases considerably. The chlorophyll content reaches its maximum after 4–6 days in the light.

3. In whole leaves in sterile culture chlorophyll synthesis progresses in about the same pattern and at the same rate as in leaf discs.

4. When etiolated intact plants were illuminated, chlorophyll synthesis started immediately at a high rate, and continued at a much higher rate than in the isolated leaves and discs. Moreover, in whole plants the chlorophyll content reached a much higher final value. Glucose, fructose, various vitamins, inositol, auxin, kinetin and gibberellin failed to bring the rate of chlorophyll synthesis in leaf discs on the level of that in the whole plant.

5. During the light-induced development of the etiolated tissue on standard medium the number of plastids per cell increases considerably.

6. The number of chloroplasts per cell has also been determined in barley and oats. In these species light-induced greening is not accompanied by plastid replication.

7. 5-Fluorouridine (FUDR) has no effect on the number of cells per leaf disc, nor on the size of the cells.

8. FUDR inhibits both chlorophyll synthesis and the growth in diameter of the plastids. It does not inhibit the increase in chloroplast number per cell on standard medium. It has no effect at all on chlorophyll synthesis in bean, and only a very slight inhibitory effect on barley.

9. FUDR has no effect on the ultrastructure of the chloroplasts.

10. The inhibiting effect of FUDR is reversed by thymidine but not by uridine.

11. Leaf discs treated with FUDR for 4 days and exposed to  $^3\text{H}$ -thymidine after this, incorporated the thymidine into the cytoplasm of all cells, and into occasional nuclei. Most of the label disappeared after treatment with DNA-ase.

12. When leaf discs were treated with kinetin during a period of 7 days in darkness and 7 days in the light, the number of cells per disc is not affected, but the cells become very large and contain large numbers of chloroplasts.

FUDR inhibits the expansion of the cells to some extent and completely abolishes the extra increase in chloroplast number per cell.

13. Nalidixic acid had about the same effect as FUDR on chloroplast ultrastructure, chloroplast numbers per cell, chloroplast size and chlorophyll synthesis.

14. It is concluded that the rate of chlorophyll synthesis in tobacco is dependent on the quantity of functional DNA.

15. It is concluded that the increase in chloroplast size depends on the amount of functional DNA.

16. It is suggested that under normal circumstances the maximum number of chloroplasts in the cell is limited by cell size, and that the effect of kinetin and auxin on this number is brought about by an increase in cell size.

17. It is suggested that chloroplasts contain more than one copy of their DNA, and that when they divide in the presence of FUDR the number of copies per plastid is reduced, causing these plastids to remain smaller than plastids with the normal number of copies, because of a gene-dosage effect.